Chemical mixtures: challenges for research and risk assessment?
Mixture identification as a first step towards hazard characterization

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Risk assessment

Single chemical

✓ BPA, MeHg, Pb, Acrylamide, Ochratoxine A,...

Mixtures?

Mixtures: based on dose/effect additivity, no interaction

✓ Chemical families: Dioxins and PCB-DL, Triazoles, Organophosphates,...

Mixtures based on toxicological data
Dietary exposure

Individuals are exposed to various substances

In the French Total Diet Study 2 (TDS 2 Anses, 2011), the following substances were searched:

~ 200 Pesticides
~ 30 Inorganic contaminants and minerals
~ 10 Additives
~ 10 Phyto-oestrogens
~ 50 Dioxins, PCB, furans, brominated flame retardants, perfluoroalkyl acids
~ 20 Mycotoxins

Multitude of possible chemical combinations for which it is unrealistic to test all possible combined effects!
Challenges

For exposure assessors:
Which are the chemicals simultaneously present in the diet and at what concentration?

✓ Define mixtures from observed exposures
✓ Propose a methodology to identify mixtures

Mixtures based on exposure data

For toxicologists:
Which are the possible effects of these mixtures?
Testing the potential effects of mixtures of pesticides to which the population is exposed

1. Characterization of pesticide mixtures
   - Estimation of combined exposures to pesticides
   - Statistical developments to identify main mixtures

2. Characterization of the combined effects of mixtures
   - Different toxicological tests to examine possible effects (cytotoxicity, real-time cell impedance, genotoxicity, oxidative stress apoptosis and PXR nuclear receptor transactivation)
   - Different human cell strains (colon, liver, kidney, brain, intestine)
   - Mixtures effects / Dose-Effect additivity of single components

Crépet A., et al. (2013) The PERICLES research program: an integrated approach to characterize the combined effects of mixtures of pesticides residues to which the French population is exposed. (Toxicology 313 2-3 83-93).
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* Crépet A., et al. (2013) The PERICLES research program: an integrated approach to characterize the combined effects of mixtures of pesticides residues to which the French population is exposed. (Toxicology 313 2-3 83-93).
Methodologies to identify mixtures from combined exposure
Dietary co-exposure assessment

For each chemical \( p \) and each individual \( i \) combine

- the consumed quantity \( Q_{i,a} \) of food \( a \) with
- the chemical concentration \( C_{p,a} \) in food \( a \)

sum on all the food \( A_p \) and then divide by the body weight \( bw_i \)

\[
E_{p,i} = \frac{\sum_{a=1}^{A_p} Q_{i,a} C_{p,a}}{bw_i}
\]

For acute exposure:

- \( Q_{i,a} \): consumed quantity per day (or maximal quantity)
- \( C_{p,a} \): a high percentile of the concentration distribution

For chronic exposure:

- \( Q_{i,a} \): mean consumed quantity per day (estimated from 7 days) or per week
- \( C_{p,a} \): mean concentration
Concentrations of food products by P chemicals, Consumption of food products by n individuals, Combined Exposure.

Individuals i=1,..,n → Exposure groups G1, G2, G3 → Combined Exposure Chemicals p=1,…,P → Combined Exposure

Mixtures:
- Mixture 1
- Mixture 2
- Mixture 3
- Mixture 4
- Mixture 5

Dietary groups G1, G2, G3 → Combined Exposure

Toxicological information?

Food vectors of mixtures?
Concentrations of food products by P chemicals consumption among n individuals.

Combined Exposure of individuals i=1,…,n to chemicals p=1,…,P.

Exposure groups:
- G1
- G2
- G3

Dietary groups:
- G1
- G2
- G3

Mixtures:
- Mixture 1
- Mixture 2
- Mixture 3
- Mixture 4
- Mixture 5

Toxicological information about the combined exposure.
Co-exposures to two pesticides

In real life: more than 2 chemicals
Define combinations from a large number of chemicals
Methods to identify mixtures

Criteria
✓ High correlations between mixture components
✓ High exposure to mixture components
✓ Large number of individuals exposed

Challenges of statistical methods
✓ Deal with large datasets (150 chemicals, >2000 individuals)
✓ Reduce the co-exposure /diet dimension to extract exposure systems/consumption systems (Factorization of Non Negative Matrix)
✓ Cluster individuals in homogenous subgroups (classification/clustering)

Publications
✓ Crépet A. et al. (2011). Bayesian nonparametric model for clustering individual co exposure to pesticides found in the French diet. (Bayesian Analysis 6(1), 127:144)
✓ Béchaux C. et al. (2013) Identification of pesticide mixtures and connection between combined exposure and diet. (Food Chemical and Toxicology 59, 191-198)
Mixtures from acute exposures to 79 pesticides

Dataset: Acute exposure
Consumption: INCA 2 (2006), French food consumption survey of 2624 adults and 1455 children
Concentrations: Monitoring programmes (2006): 79 pesticides

<table>
<thead>
<tr>
<th>Mixture 1</th>
<th>Mixture 2</th>
<th>Mixture 3</th>
<th>Mixture 4</th>
<th>Mixture 5</th>
<th>Mixture 6</th>
<th>Mixture 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphenylamine</td>
<td>Linuron</td>
<td>Fenitrothion</td>
<td>Lambda-Cyhalothrin</td>
<td>DDT</td>
<td>Maleic hydrazide</td>
<td>Imazalil</td>
</tr>
<tr>
<td>Tolylfluanid</td>
<td>Chlorfenvinphos</td>
<td>Quinoxyfen</td>
<td>Iprodione</td>
<td>Dieldrin</td>
<td>Chlorpropham</td>
<td>Methidathion</td>
</tr>
<tr>
<td>Phosalone</td>
<td>Ethion</td>
<td>Fenhexamid</td>
<td>Procymidone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propargite</td>
<td>Triadimenol</td>
<td>Cyprodinil</td>
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<td></td>
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</tr>
<tr>
<td>Captan</td>
<td>Pyrimethanil</td>
<td>Fludioxonil</td>
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<td></td>
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<tr>
<td></td>
<td>Penconazole</td>
<td></td>
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</tr>
</tbody>
</table>

25 pesticides in 7 mixtures
Mixtures combining 2 to 6 pesticides from different chemical families

Concentrations of food products by P chemicals
Consumption of food products by n individuals

Combined Exposure

Dietary groups
- G1
- G2
- G3

Chemicals
- p=1,...,P

Exposure groups
- i=1,...,n

Mixtures
- Mixture 1
- Mixture 2
- Mixture 3
- Mixture 4
- Mixture 5

Toxicological information
Diet contribution

Individuals have particular food behaviors

Define cluster diets

 ✓ Major food combinations (consumption systems)
 ✓ Subgroups of individuals with similar consumption profiles

Identify main mixtures associated to these food combinations

Datasets: Chronic exposure

 ✓ Consumption: 212 foods from INCA2, 2624 adults
 ✓ Concentrations:
   ✓ 26 pesticides in Total Diet Study 2
   ✓ 149 substances in Total Diet Study 2: Additives, Inorganic contaminants & minerals, Acrylamide, BPA, PAHs, Mycotoxins, Dioxins, Furans, PCBs, Perfluorinated compounds, Pesticides, Phytoestrogens
Mixtures from chronic exposures to 26 pesticides

6 groups of individuals combining 1 or 2 of the 6 major consumption systems (CS) and therefore exposed to 1 or 2 mixtures among the 6 major mixtures

<table>
<thead>
<tr>
<th>Group</th>
<th>% of CS</th>
<th>Major Consumption Systems</th>
<th>Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2</td>
<td>37%</td>
<td>Vegetable soup, Apple, Endive, Carrot, Kiwi, Lettuce, etc</td>
<td>Imazalil, Pirimiphos-methyl</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>14%</td>
<td>Beef steak, Pasta, Baguette, Cooked ham, Butter, Egg, Coffee, etc</td>
<td>Endosulfan, Dichlorvos, Pirimiphos-methyl, Caborfuran</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 6</td>
<td>51%</td>
<td>Pizza, Hamburger, Soda, Sandwich, Sauteed potatoes or chips Instant hot chocolate drink</td>
<td>Carbofuran, Chlorpyrifos-ethyl, Dichlorvos, Endosulfan</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mayonnaise, etc</td>
<td>Phosmet</td>
</tr>
<tr>
<td></td>
<td>21%</td>
<td>Beef steak, Pasta, Baguette, Cooked ham, Butter, Egg, Coffee, etc</td>
<td>Endosulfan, Dichlorvos, Pirimiphos-methyl, Caborfuran</td>
</tr>
</tbody>
</table>

*significantly different from the whole population

Mixtures from chronic exposures to 149 substances

6 groups of individuals combining 1 or 2 of the 6 major consumption systems (CS) and therefore exposed to 1 or 2 mixtures among the 6 major mixtures

<table>
<thead>
<tr>
<th>Group</th>
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<th>Major Consumption Systems</th>
<th>Mixtures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2</td>
<td>37%</td>
<td>Vegetable soup, Apple, Endive, Carrot, Kiwi, Lettuce, etc.</td>
<td>Ba, Pb, Al, Sb, Cd Thiabendazole, Diphenylamine, Porpargite</td>
</tr>
<tr>
<td>N=708</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age=53*</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BMI=24.3</td>
<td></td>
<td></td>
<td>HT2, Don, Niv, Zer (Mycotoxins), BPA, PY, PHE (PAHs) Cd, Co, Te</td>
</tr>
<tr>
<td>%Women=65*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14%</td>
<td>Beef steak, Pasta, Baguette, Cooked ham, Butter, Egg, Coffee, etc.</td>
<td>PHE, PY, FA (PAHs), Zer (Mycotoxins), Pirimiphos-methyl PBDEs Co, Nitrites, Acrylamid</td>
<td></td>
</tr>
<tr>
<td>Group 6</td>
<td>51%</td>
<td>Pizza, Hamburger, Soda, Sandwich, Sauteed potatoes or chips Instant hot chocolate drink Mayonnaise, etc.</td>
<td>HT2, Don, Niv, Zer (Mycotoxins), BPA, PY, PHE (PAHs) Cd, Co, Te</td>
</tr>
<tr>
<td>N=303</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age=28*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI=22.3*</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>%Men=51%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21%</td>
<td>Beef steak, Pasta, Baguette, Cooked ham, Butter, Egg, Coffee, etc.</td>
<td></td>
<td></td>
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*significantly different from the whole population

Publication in progress
Concentrations
F food products by
P chemicals

Consumption
F food products by
n individuals

Combined Exposure

Individuals
i=1,…,n

Chemicals
p=1,…,P

Dietary groups

Concentrations

G1

G2

G3

Mixtures

Mixture 1
Mixture 2
Mixture 3
Mixture 4
Mixture 5

Exposure groups

G1

G2

G3

Toxicological information

Combined Exposure

Combined Exposure

Combined Exposure

Combined Exposure
Integrating toxicological information to identify mixtures

Availability of toxicological data?
- Data base: Well adapted and completeness
- Need literature review

Type of data to integrate?
- Target organ
- Toxicological effect
- Mode of action
- The one related to the reference value

Only for single component

Is this data selective enough?

Dataset: non exhaustive toxicological information for the 149 substances: trial with the 73 pesticides
Trial with the 73 pesticides

Efsa report 2013 « Toxicological data for grouping pesticides »
- 3 effects: hepatotoxicity, reprotoxicity, and neurotoxicity
- 3 responses: Y(effect), N(no effect), NA (not available)

Only the neurotoxic effect was selective
All pesticides for which information was available were hepatotoxic and reprotoxic

Among the 73 pesticides, for the neurotoxic effect
- 13 with the effect
- 8 with no effect
- 52 with no information
Trial with the 73 pesticides

Multiple Factor analysis (MFA) to balance exposure and toxicological data

**Benefit:** new mixtures built considering both exposure and toxicological information

**Drawback:** statistical method not well adapted, classification on pesticides based on non homogeneous individual exposures (high and low exposed individuals)
Integrated approach to improve exposure assessment to mixtures

Dynamic modeling of Aggregated Exposure, PBPK model, biomonitoring data
Dietary Exposure

Exposure from Air

Exposure from Dust

Identify mixtures

Aggregated exposure

Body d

Level at biof

Chemical Kinetic

TK, PBTK model for mixtures

Biomonitoring data

In progress: Camille Béchaux, PhD Student (2011-2014)
Conclusions

The proposed methodologies lead to identify mixtures
- Based on observed combined exposures
- Regardless the chemical family
- Considering and identifying dietary habits
- Identifying populations exposed to specific mixtures

Identify mixtures to prioritize
- Hazard characterization (toxicological tests, PBTK, TD models)
- Mixtures risk assessment
Perspectives

Mixtures identification
✓ To identify mixtures at aggregated level
✓ To identify mixtures from biomonitoring data
✓ To better integrate toxicological information which requires more solid toxicological data and improvements in statistics

To develop an integrated approach for mixtures
PERICLES team and al.

Anses-DER: Camille Béchaux, Jean-Charles Leblanc, Fanny Héraud (Efsa), Thiéma Traore

Anses-DIVE: Marie Estelle Gouze, Suzanne Pierlot, Antony Fastier

Anses-Fougères: Ludovic Le Hégarat, Natsuko Takakura, Valérie Fessard

INRA: Jessica Tressou, Moustapha Sy (CEA), Max Feinberg, Nawaz, A., Georges de Sousa, Nathalie Zucchiini-Pascal, Roger Rahmani, Marc Audebert, Vanessa Graillot, Jean Pierre Cravedi

TelecomParisTech: Stéphan Clémençon

WHO: Philippe Verger

CEA: Rémi Maximilien

Thank you for your attention